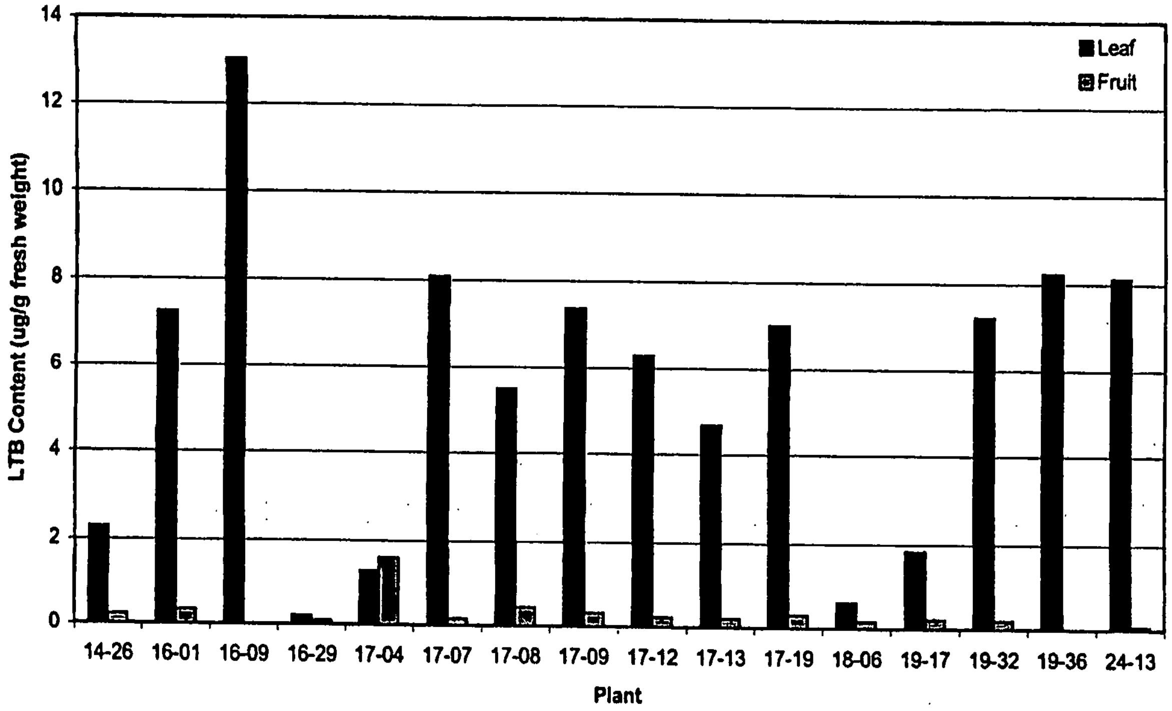

14
12
10
8
6
4
2
0
LTB Content (ug/g fresh weight)
■ Leaf
▨ Fruit
14-26
16-01
16-09
16-29
17-04
17-07
17-08
17-09
17-12
17-13
17-19
18-06
19-17
19-32
19-36
24-13
Plant

Figure 1a

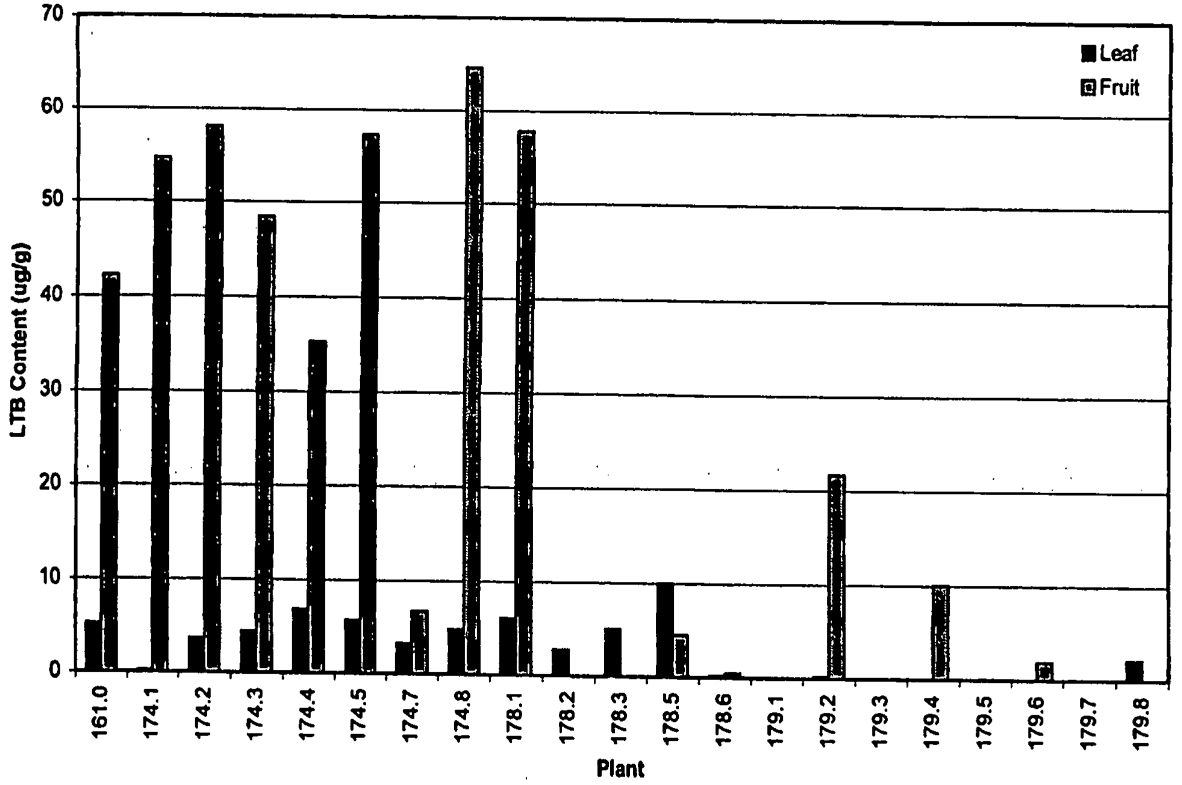

70
60
50
40
30
20
10
0
LTB Content (ug/g)
Leaf
Fruit
161.0
174.1
174.2
174.3
174.4
174.5
174.7
174.8
178.1
178.2
178.3
178.5
178.6
179.1
179.2
179.3
179.4
179.5
179.6
179.7
179.8
Plant

Figure 1b

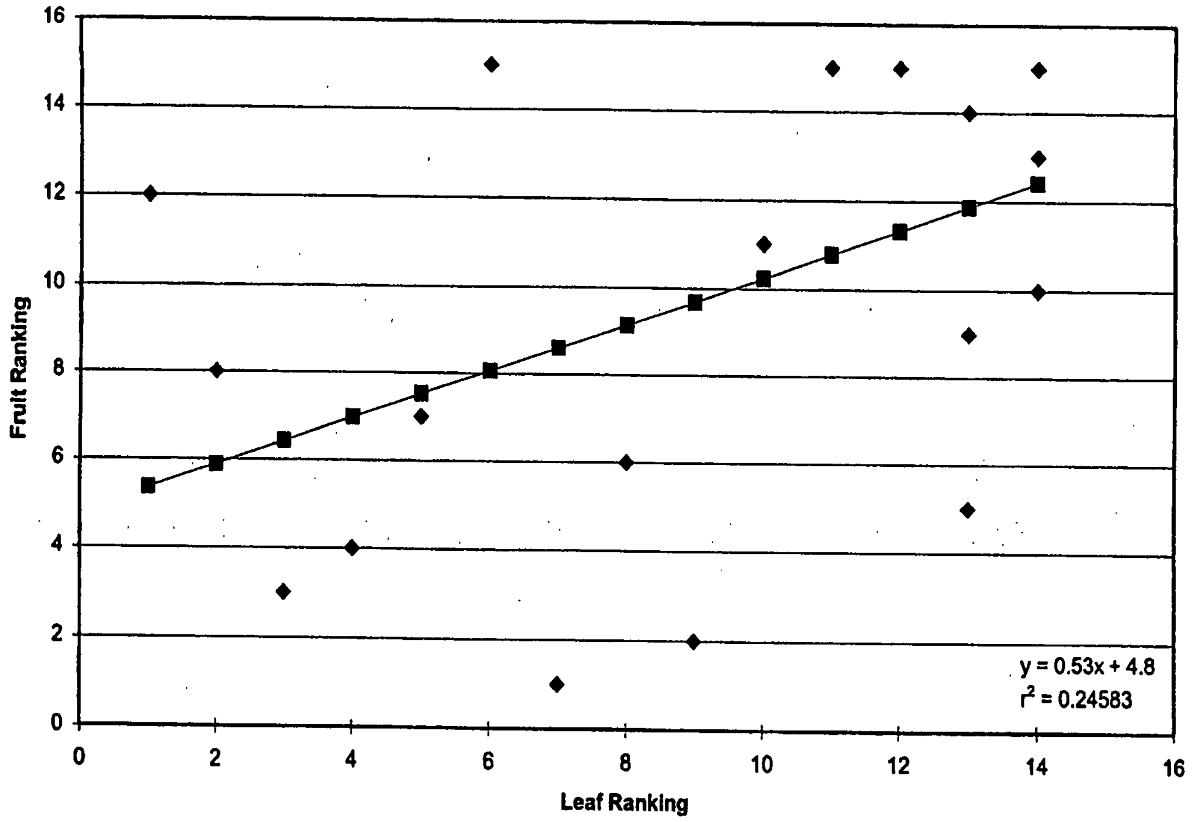

16
14
12
10
8
6
4
2
0
Fruit Ranking
0
2
4
6
8
10
12
14
16
Leaf Ranking
y = 0.53x + 4.8
r² = 0.24583

Figure 1 c

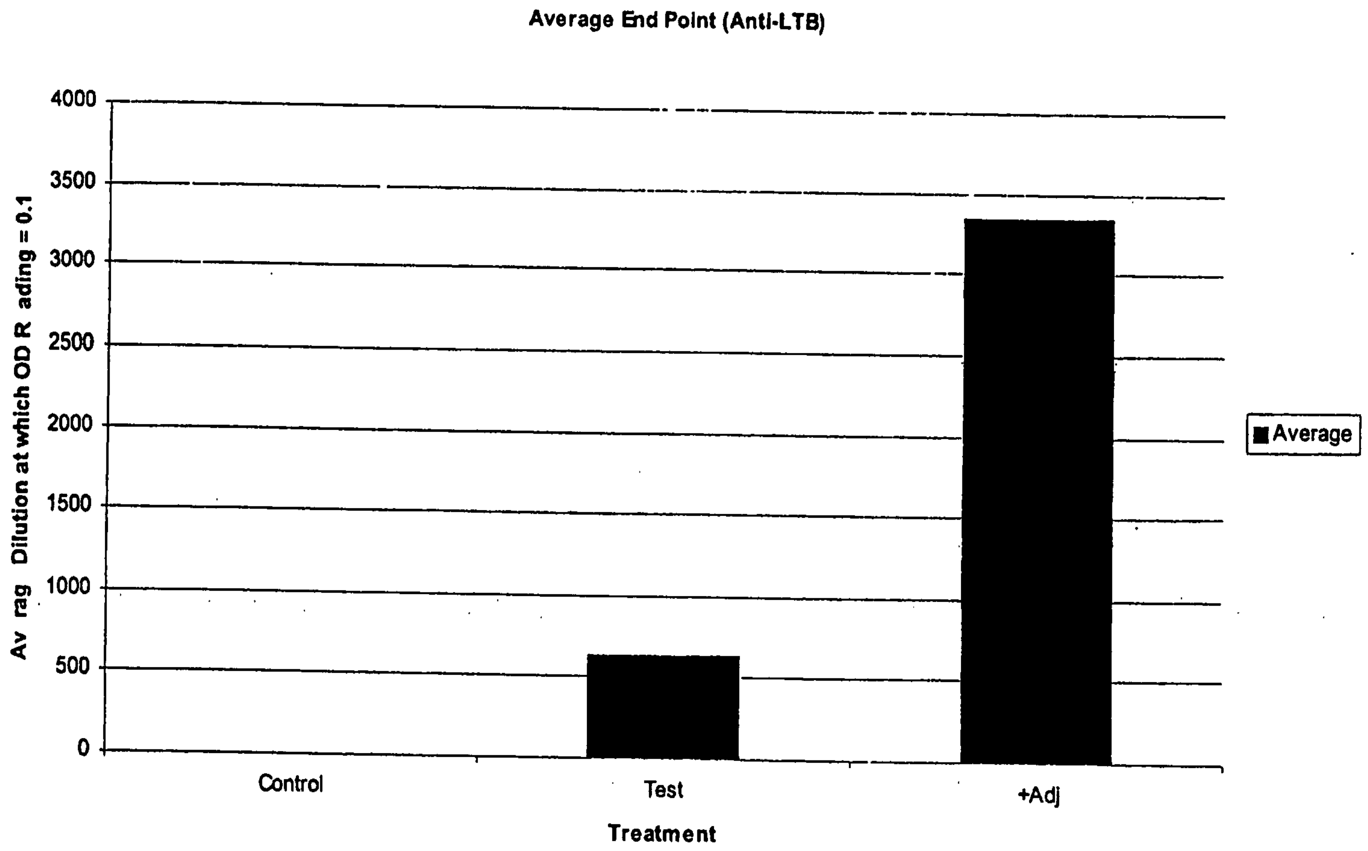

Average End Point (Anti-LTB)
Av rag Dilution at which OD R ading = 0.1
4000
3500
3000
2500
2000
1500
1000
500
0
Control
Test
+Adj
Treatment
Average

Figure 2a

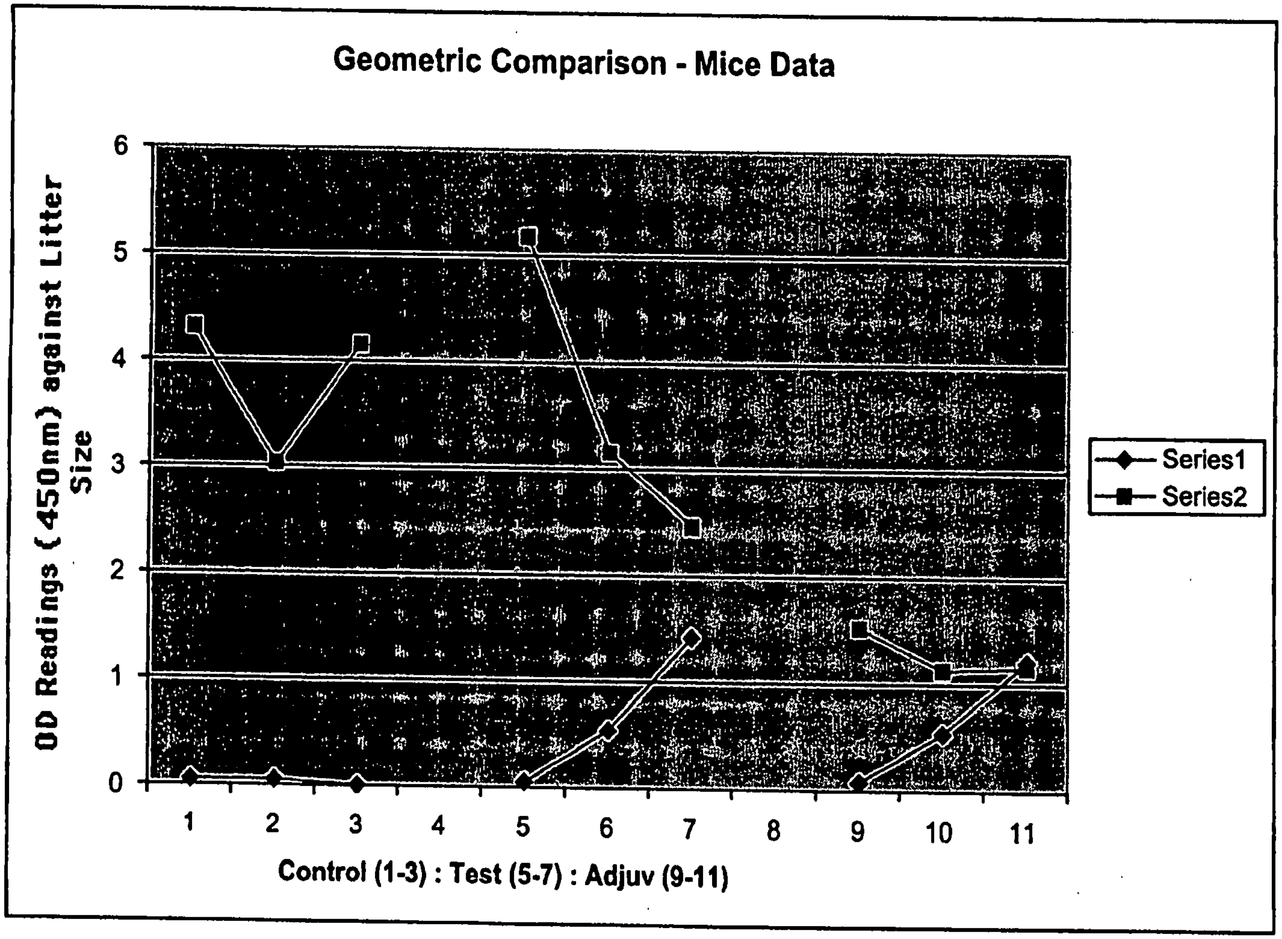

Geometric Comparison - Mice Data
OD Readings (450nm) against Litter Size
0
1
2
3
4
5
6
1
2
3
4
5
6
7
8
9
10
11
Control (1-3) : Test (5-7) : Adjuv (9-11)
Series1
Series2

Figure 2 b

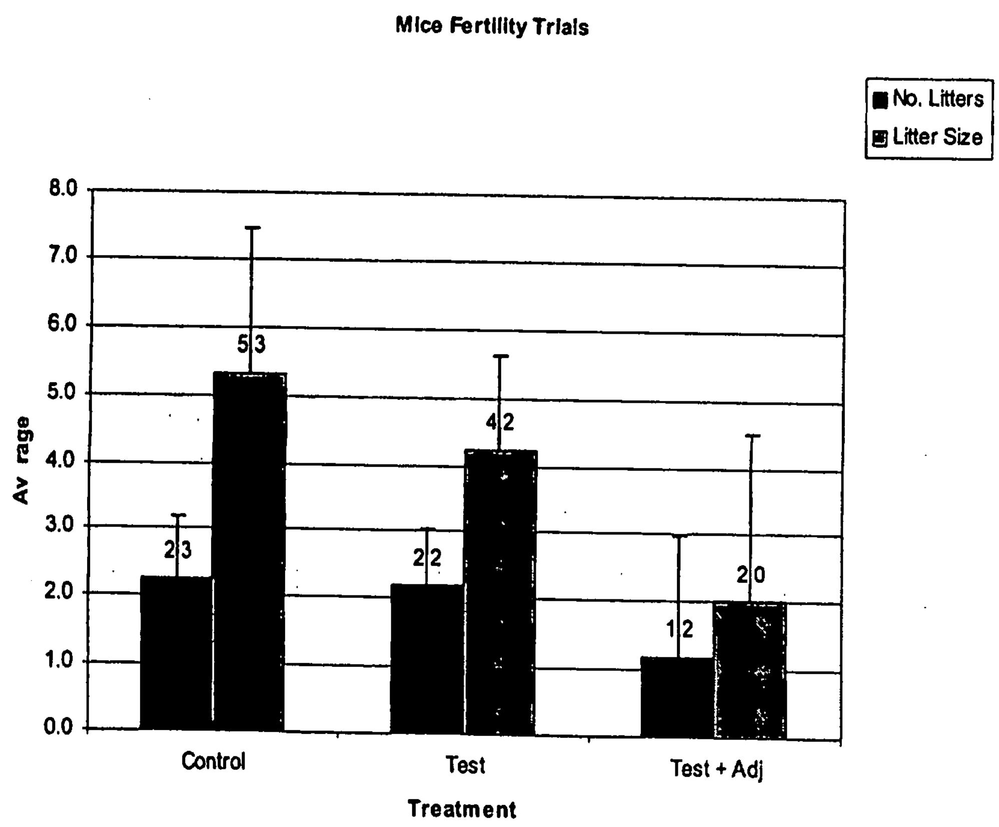

Mice Fertility Trials
■ No. Litters
▨ Litter Size
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Av rage
2.3
5.3
2.2
4.2
1.2
2.0
Control
Test
Test + Adj
Treatment

Figure 3.

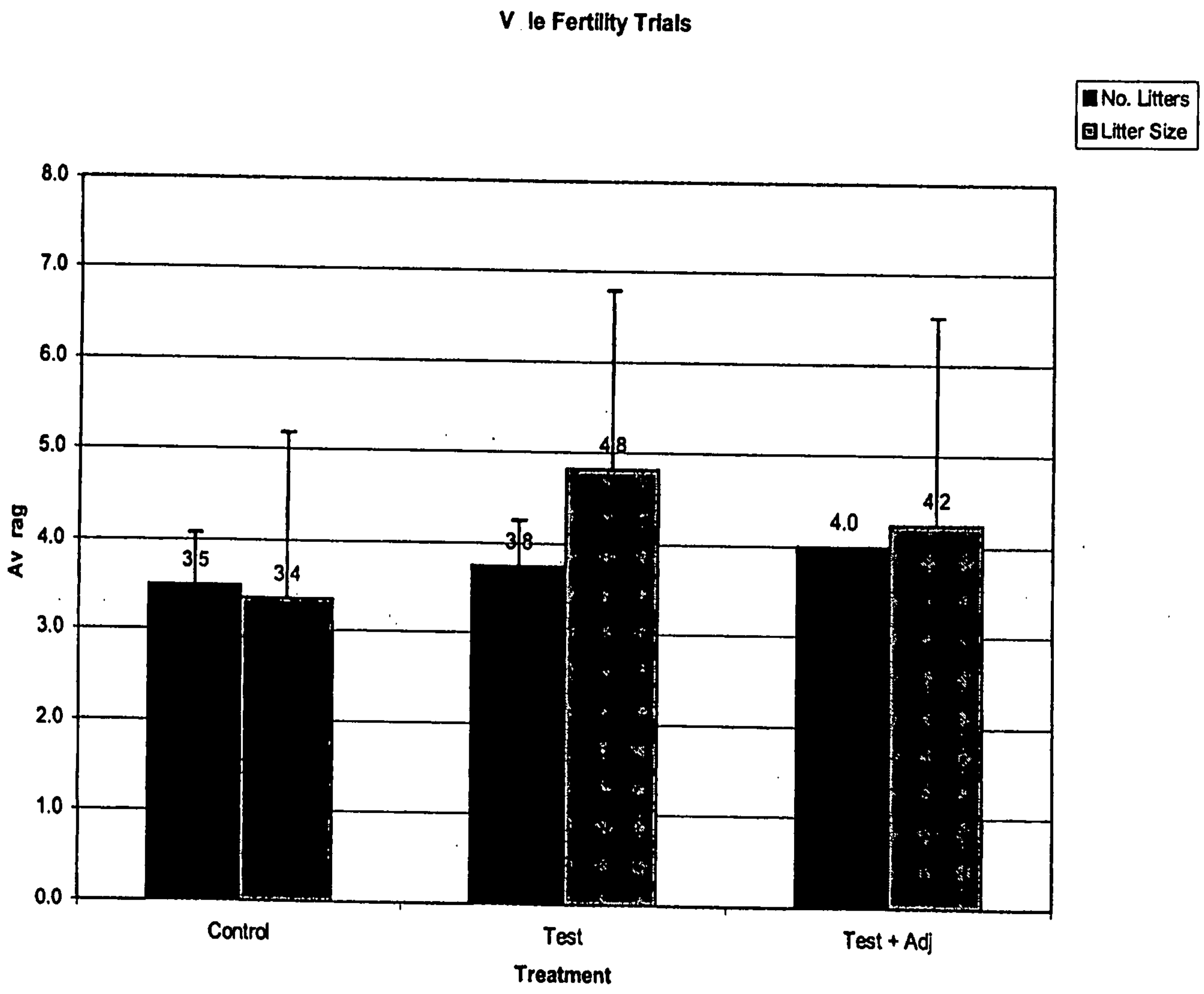

Vole Fertility Trials
■ No. Litters
⊡ Litter Size
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Av rag
3.5
3.4
3.8
4.8
4.0
4.2
Control
Test
Test + Adj
Treatment

Figure 4

Figure 5 a Contraceptive - LTB Fusion Constructs

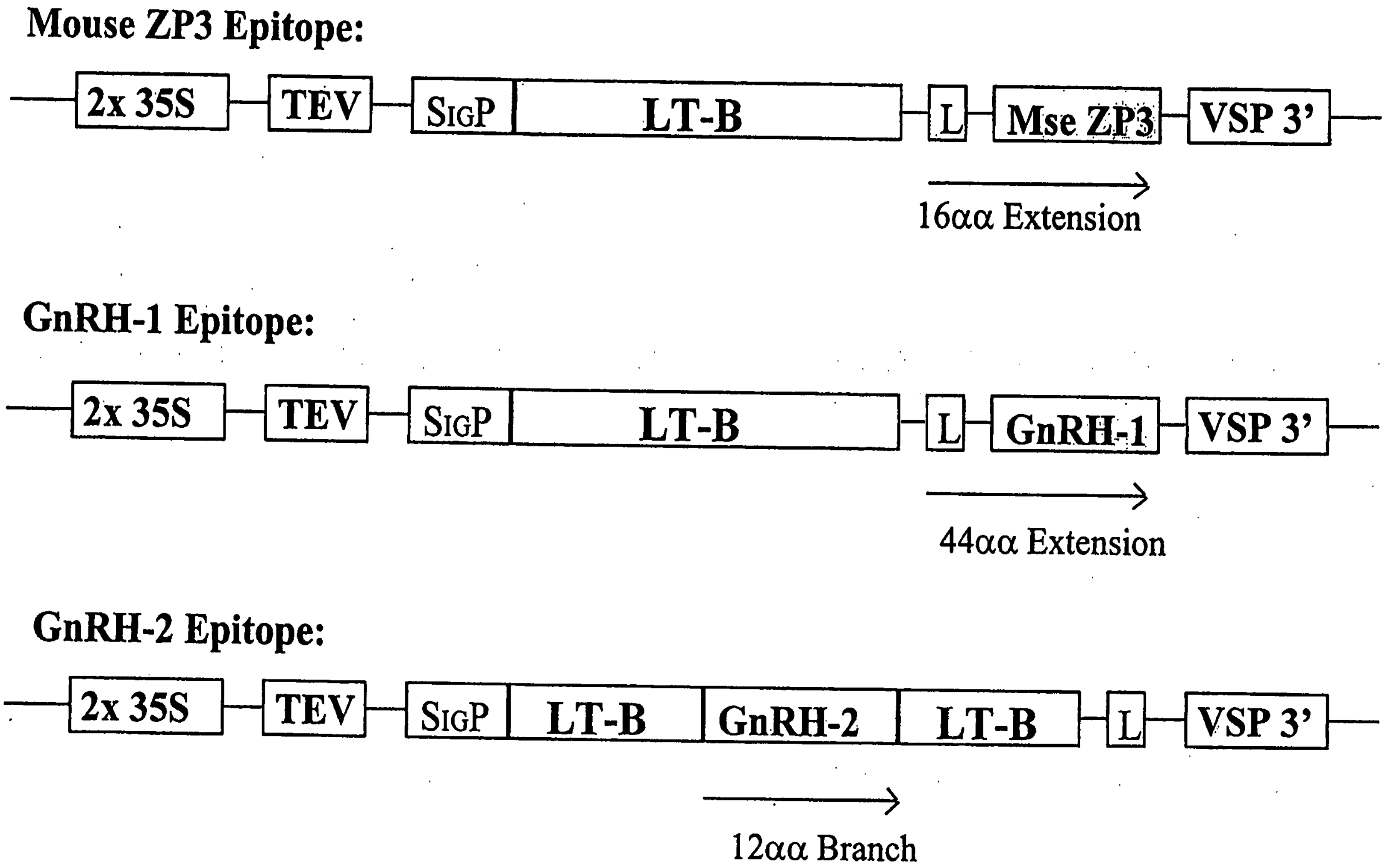
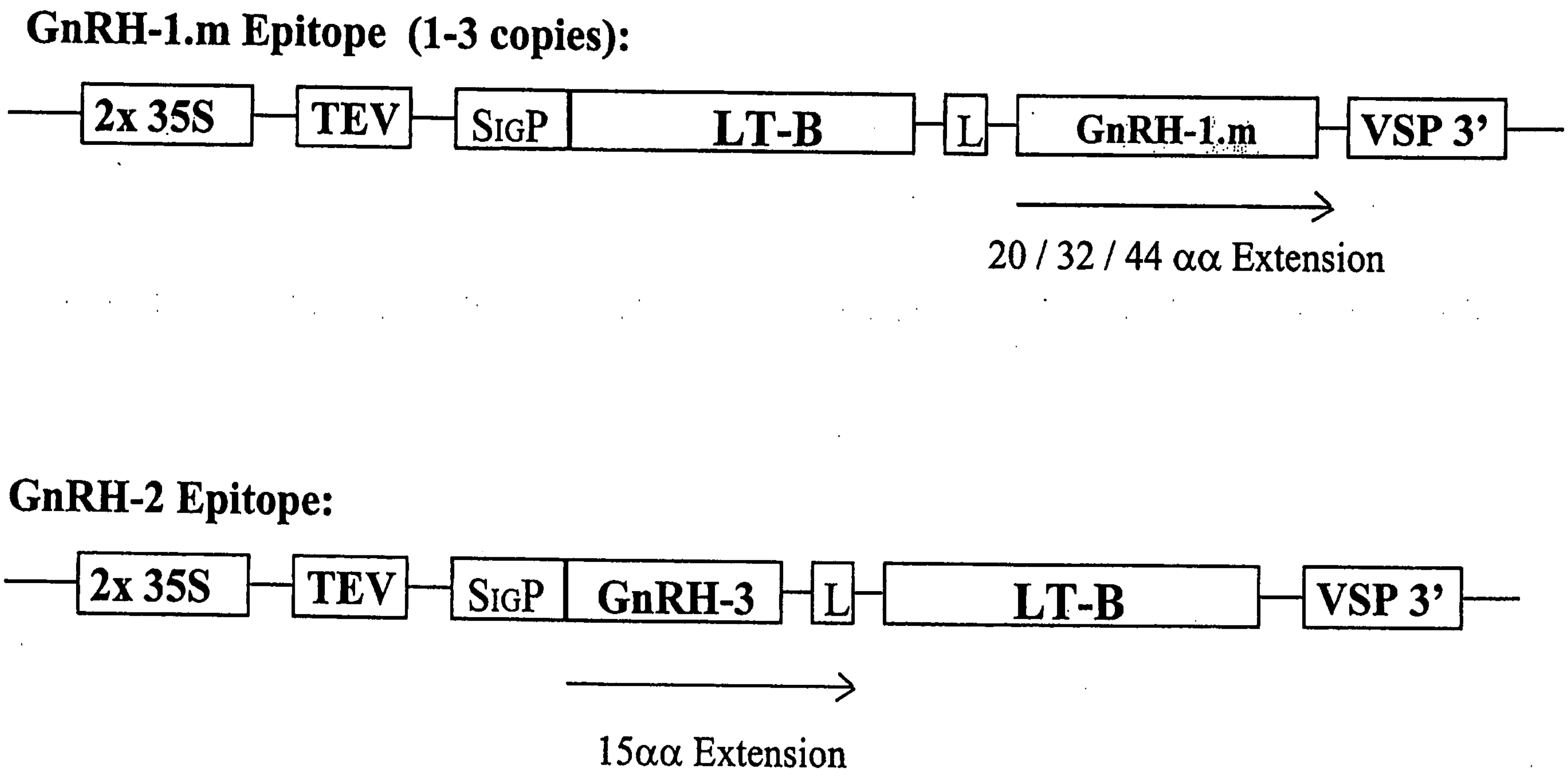
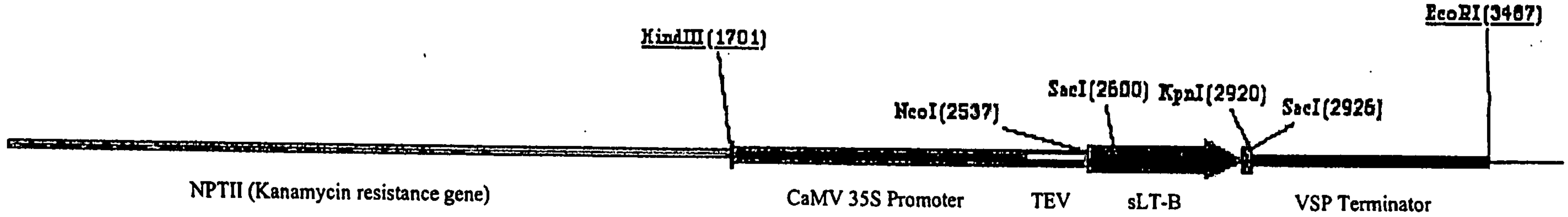
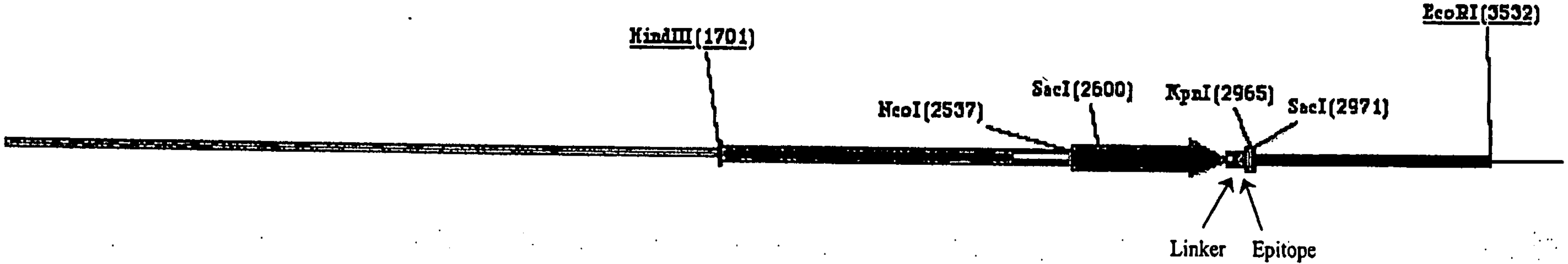


Figure 5 b Contraceptive - LTB Fusion Constructs





Synthetic LT-B cassette
(3658 bp)



C-terminal fusion cassette
(3703 bp)

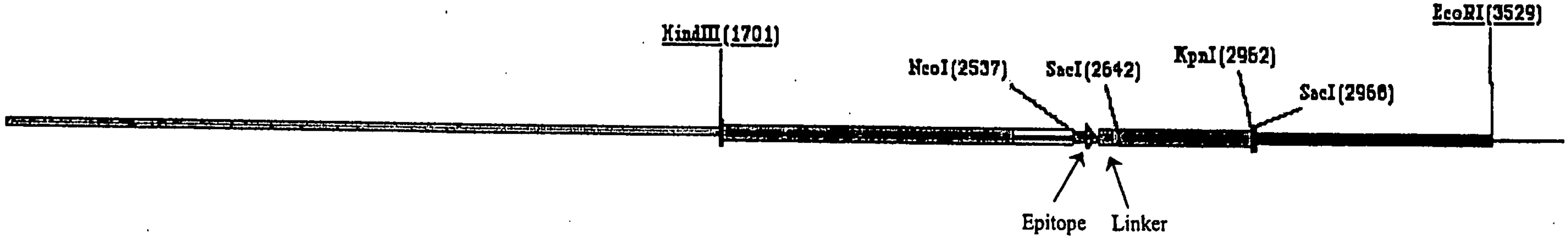


Figure 5 c N-terminal fusion cassette (3700 bp)

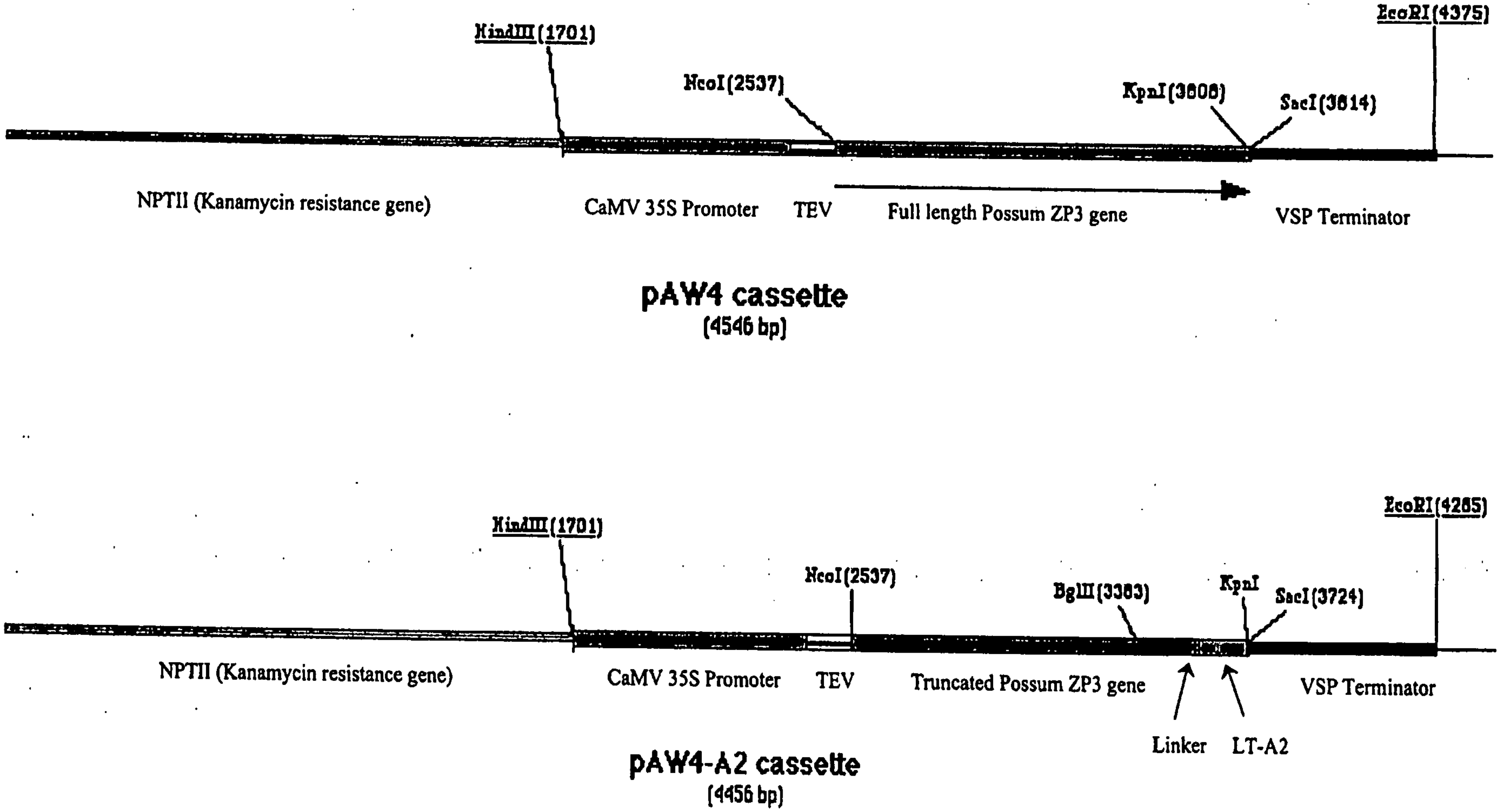


Figure 5 c N-terminal fusion cassette (3700 bp) continued

Fusion Construct	Total Soluble Protein Conc.	Average Level of LT-B Detected	Highest Level of LT-B Detected	Highest Level of Epitope Detected
LTB-Mouse ZP3	16.5ug/g FW	0.74ug/g FW	2.90ug/g FW	50ng/g FW
LTB-GnRH1	17.3ug/g FW	0.61ug/g FW	1.21ug/g FW	270ng/g FW
LTB- GnRH2	14.5ug/g FW	0.22ug/g FW	0.29ug/g FW	0ng/g FW

Figure 6

Processed Plant Type	Antigen of Interest	Post Processing Treatment Group	Sample 1 Antigen Content	Sample 2 Antigen Content	Sample 3 Antigen Content	Average Antigen Content	Greatest Variance
Tomato fruit	HBsAg combined	Frozen	89.9	92.4	n/a	91.1	**3%**
		Room Temp	76.1	90.8	81	82.6	**19%**
Tomato fruit	HBsAg combined	Frozen	19.8	23.1	n/a	21.5	**17%**
		Room Temp	15.5	15.5	19.4	16.8	**25%**
		RT (+antioxidant)	13.2	11.4	12.8	12.5	**16%**
		Frozen (+antioxidant)	26.8	28.2	n/a	27.5	**5%**
Tomato fruit	NVCP combined	Frozen	1.34	1.49	n/a	1.4	**11%**
		Room Temp	1.42	1.68	n/a	1.6	**18%**
		RT (+adjuvant)	1.22	1.13	n/a	1.2	**8%**
		Frozen (+adjuvant)	0.95	0.93	n/a	0.9	**2%**
Potato tuber	NVCP combined	None	16.4	17.1	15.2	16.2	**13%**
		+antioxidant	14.3	15.7	17.1	15.7	**20%**
		+antioxidant +adjuvant	16.8	13.9	14.9	15.2	**21%**
Potato tuber	NVCP combined	Room Temp	7.2	5.9	6.3	6.5	**22%**
		RT (+antioxidant)	4.6	5.1	3.9	4.5	**31%**
		Frozen (+antioxidant)	11.9	11.1	9.8	10.9	**21%**
		RT (+adjuvant)	5.1	5.1	3.8	4.7	**34%**
		Frozen (+adjuvant)	12.1	9.9	10.5	10.8	**22%**
Potato Tubers	NDV-HN- line 4	None	5.44	6.63	7.06	6.4	**30%**
		UNPROCESSED	0.47	2.4	0.95	1.3	**153%**
Potato Tubers	NDV-HN- line 6	None	27.87	39.43	22.62	30.0	**74%**
		UNPROCESSED	1.16	2.26	5.07	2.8	**337%**
Potato Tubers	AIV-HA- line 109	None	5.6	6.1	7	6.2	**25%**
		UNPROCESSED	1.18	1.81	1.91	1.6	**62%**
Potato Tubers	AIV-HA- line 73	None	5.6	6	6.7	6.1	**20%**
		UNPROCESSED	3.64	2.26	2.01	2.6	**81%**

TABLE 1

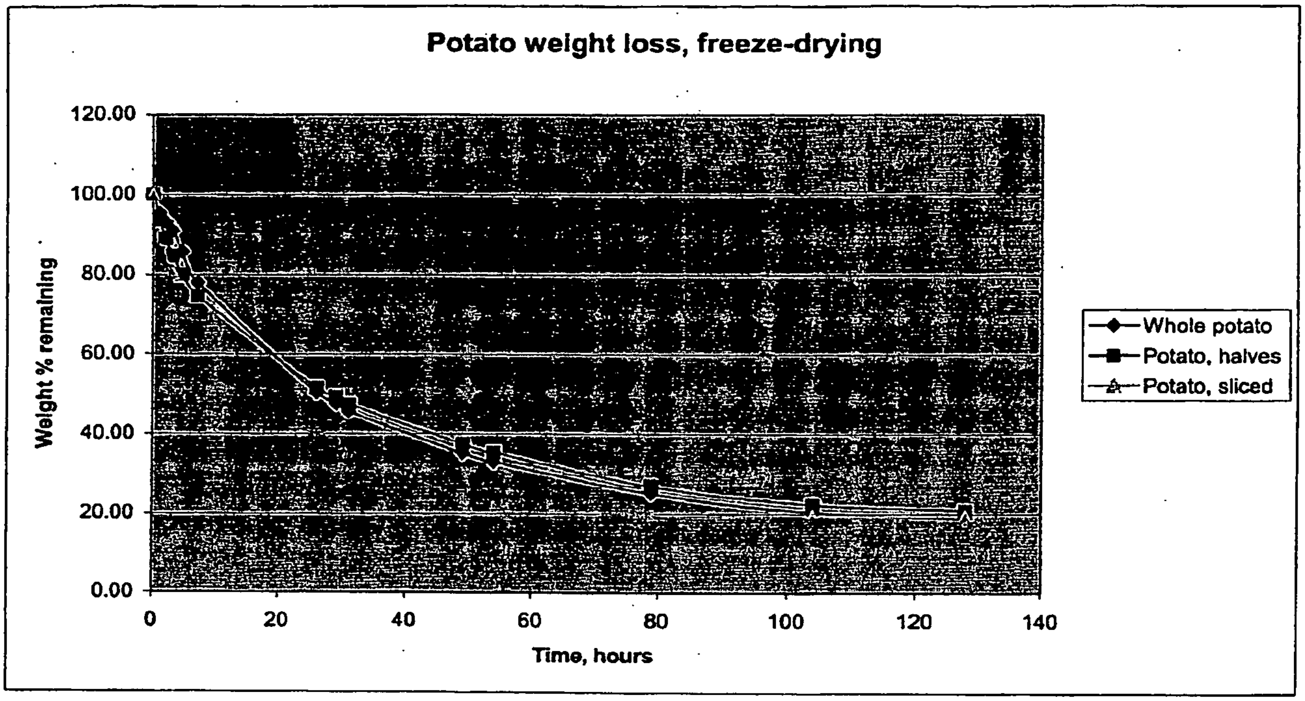

Potato weight loss, freeze-drying
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Weight % remaining
0
20
40
60
80
100
120
140
Time, hours
Whole potato
Potato, halves
Potato, sliced

FIGURE 7

Plant Source (- Antigen)	Pre-processing Method	Reduction in Mass	Antigen Recovery	Total Concentration Effect
Potato – NVCP	Puree, freeze-dry	88%	43%	3.6 fold
Potato – AIV:HA	Puree, freeze-dry	89%	52%	4.7 fold
Potato – NDV:HN	Puree, freeze-dry	83%	52%	3.1 fold
Potato – HBsAg	Puree, freeze-dry	90%	57%	5.7 fold
Potato – LTB	Puree, freeze-dry	89%	44%	4.0 fold
Tomato – NVCP	Puree, freeze-dry	94%	43%	7.2 fold
Tomato – HBsAg	Puree, freeze-dry	95%	59%	11.8 fold
Tomato – LTB	Puree, freeze-dry	94%	48%	8.0 fold
Potato – AIV:HA	Cube, freeze-dry	84%	95%	15.2 fold
Potato – NDV:HN	Cube, freeze-dry	84%	95%	15.2 fold
Carrot root – LTB	None, freeze-dry	88%	~ 100%	8.3 fold
Carrot root – ZP3	None, freeze-dry	92%	~ 100%	12.5 fold
Carrot leaf – LTB	None, freeze-dry	90%	~ 100%	10.0 fold
Carrot leaf – ZP3	None, freeze-dry	90%	~ 100%	10.0 fold

TABLE 2

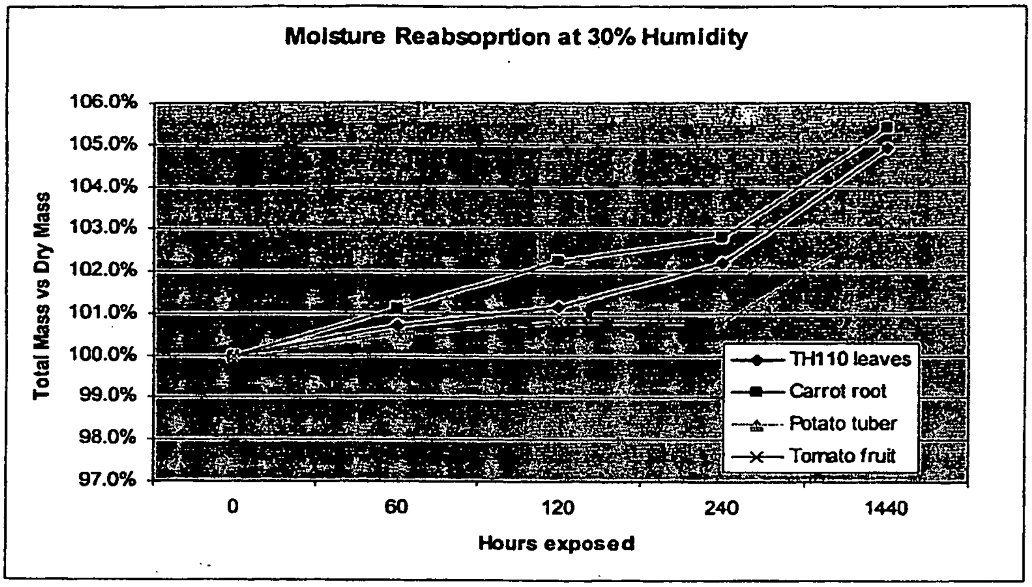
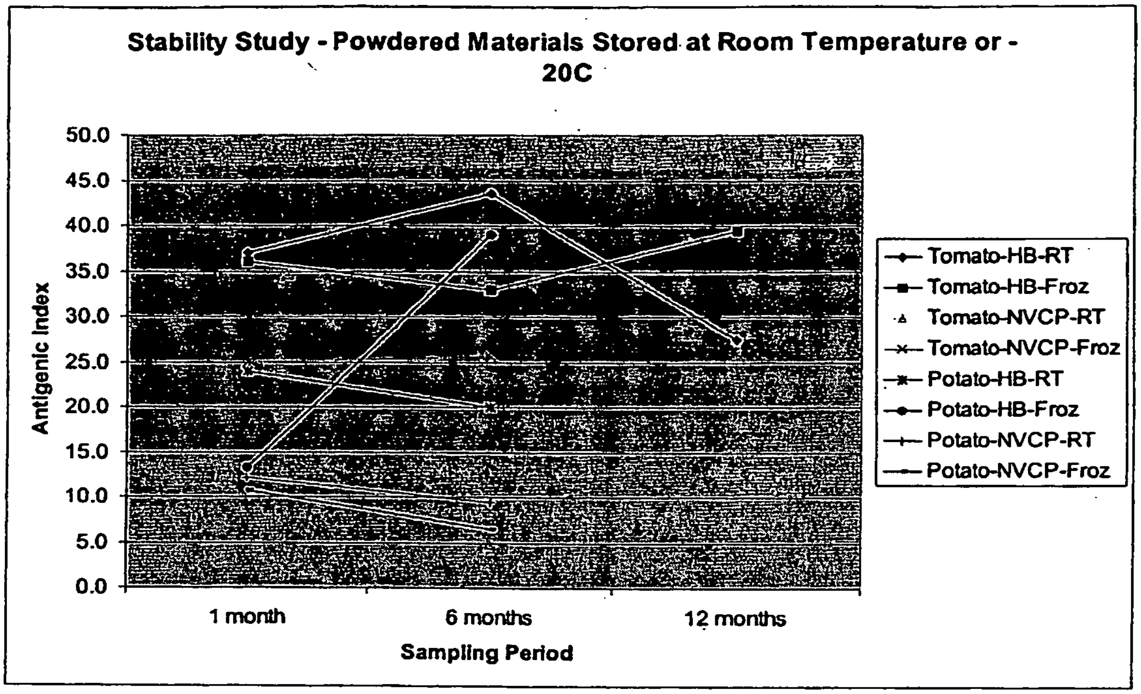

Moisture Reabsoprtion at 30% Humidity
Total Mass vs Dry Mass
106.0%
105.0%
104.0%
103.0%
102.0%
101.0%
100.0%
99.0%
98.0%
97.0%
0
60
120
240
1440
Hours exposed
TH110 leaves
Carrot root
Potato tuber
Tomato fruit

FIGURE 8



Stability Study - Powdered Materials Stored at Room Temperature or -20C
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Antigenic Index
1 month
6 months
12 months
Sampling Period
Tomato-HB-RT
Tomato-HB-Froz
Tomato-NVCP-RT
Tomato-NVCP-Froz
Potato-HB-RT
Potato-HB-Froz
Potato-NVCP-RT
Potato-NVCP-Froz

FIGURE 9

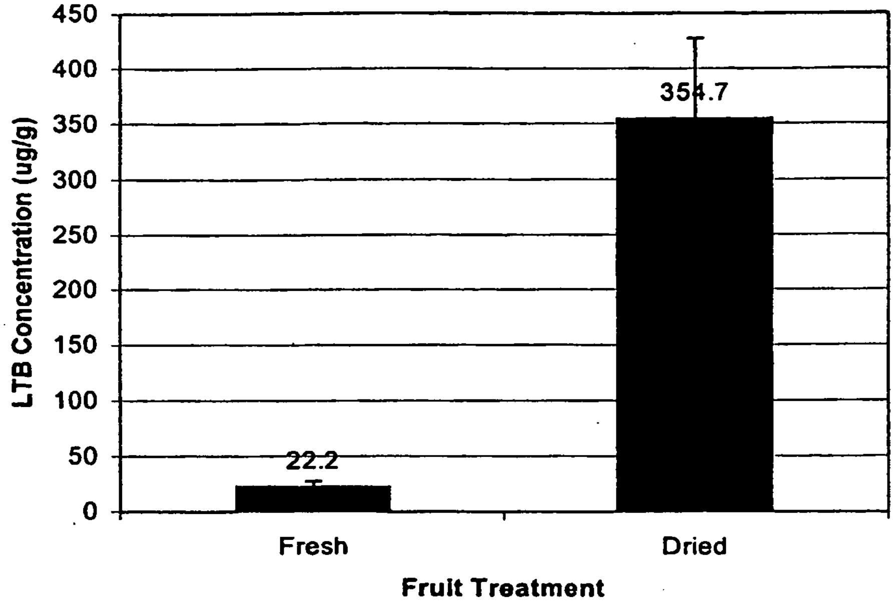
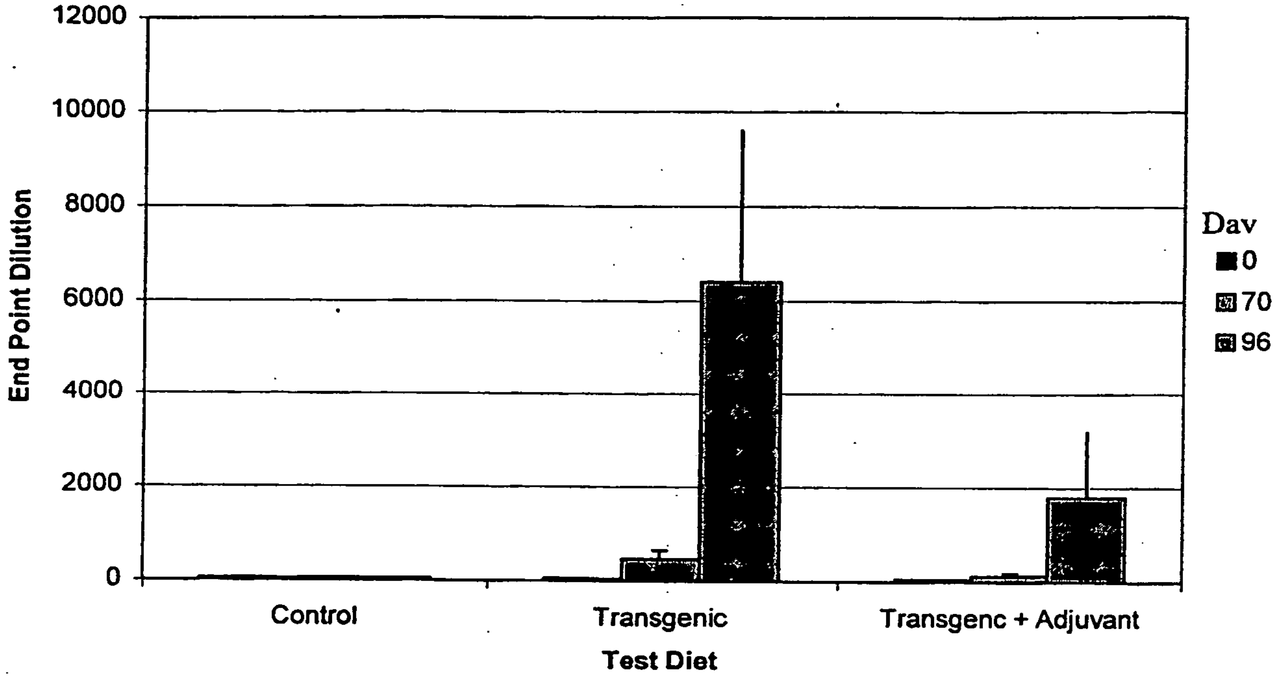

LTB Concentration (ug/g)
450
400
350
300
250
200
150
100
50
0
22.2
354.7
Fresh
Dried
Fruit Treatment

FIGURE 10



12000
10000
8000
6000
4000
2000
0
End Point Dilution
Dav
0
70
96
Control
Transgenic
Transgenc + Adjuvant
Test Diet

FIGURE 11

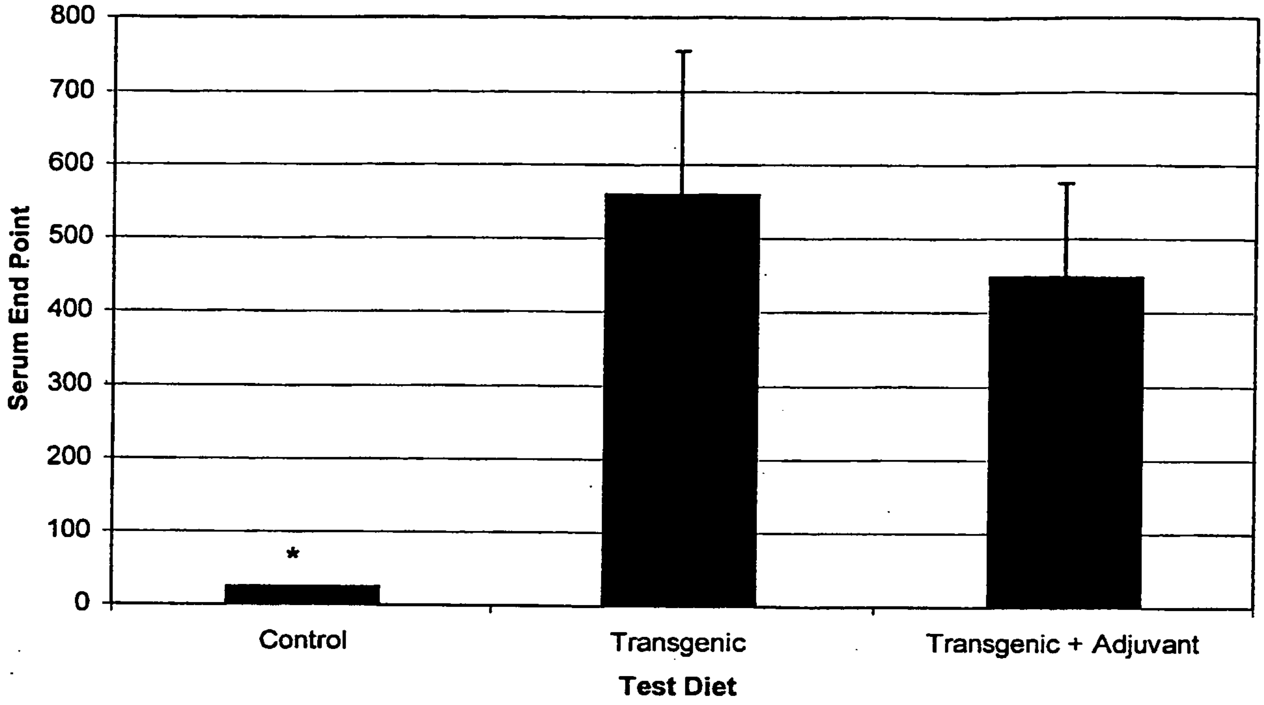

800
700
600
500
400
300
200
100
0
Serum End Point
*
Control
Transgenic
Transgenic + Adjuvant
Test Diet

FIGURE 12

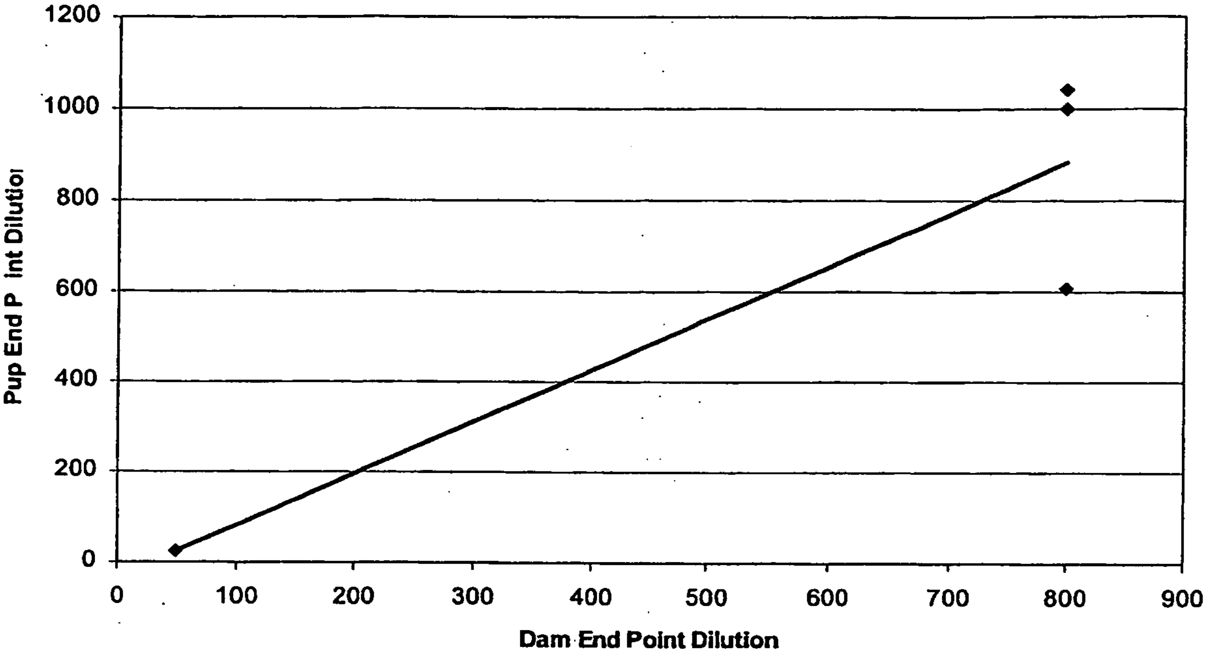

1200
1000
800
600
400
200
0
Pup End P int Dilutio
0
100
200
300
400
500
600
700
800
900
Dam End Point Dilution

FIGURE 13

Lyoph LT-B TH110 Potatoes			Lyoph LT-B TH110 Potatoes with Sodium Ascorbate			Lyoph LT-B TH110 Potatoes with Sodium Ascorbate & Quil A		
Stability Study			Stability Study			Stability Study		
	Storage	LT-B ELISA		Storage	LT-B ELISA		Storage	LT-B ELISA
12-Oct-01	(6-12°C)	32.6	12-Oct-01	(6-12°C)	42.8	12-Oct-01	(6-12°C)	26.0
12-Oct-01	(R.T.)	35.5	12-Oct-01	(R.T.)	31.9	12-Oct-01	(R.T.)	28.1
28-Nov-01	(6-12°C)	18.2	28-Nov-01	(6-12°C)	20.6	28-Nov-01	(6-12°C)	23.4
28-Nov-01	(R.T.)	23.7	28-Nov-01	(R.T.)	25.5	28-Nov-01	(R.T.)	21.8
12-Jan-02	(6-12°C)	14.7	12-Jan-02	(6-12°C)	16.9	12-Jan-02	(6-12°C)	14.4
12-Jan-02	(R.T.)	19.8	12-Jan-02	(R.T.)	22.5	12-Jan-02	(R.T.)	17.7
13-Feb-02	(6-12°C)	25.0	13-Feb-02	(6-12°C)	22.7	13-Feb-02	(6-12°C)	17.2
13-Feb-02	(R.T.)	24.4	13-Feb-02	(R.T.)	19.3	13-Feb-02	(R.T.)	15.8
14-Mar-02	(6-12°C)	21.4	14-Mar-02	(6-12°C)	24.8	14-Mar-02	(6-12°C)	23.4
14-Mar-02	(R.T.)	25.7	14-Mar-02	(R.T.)	25.3	14-Mar-02	(R.T.)	26.3

Figure 14a

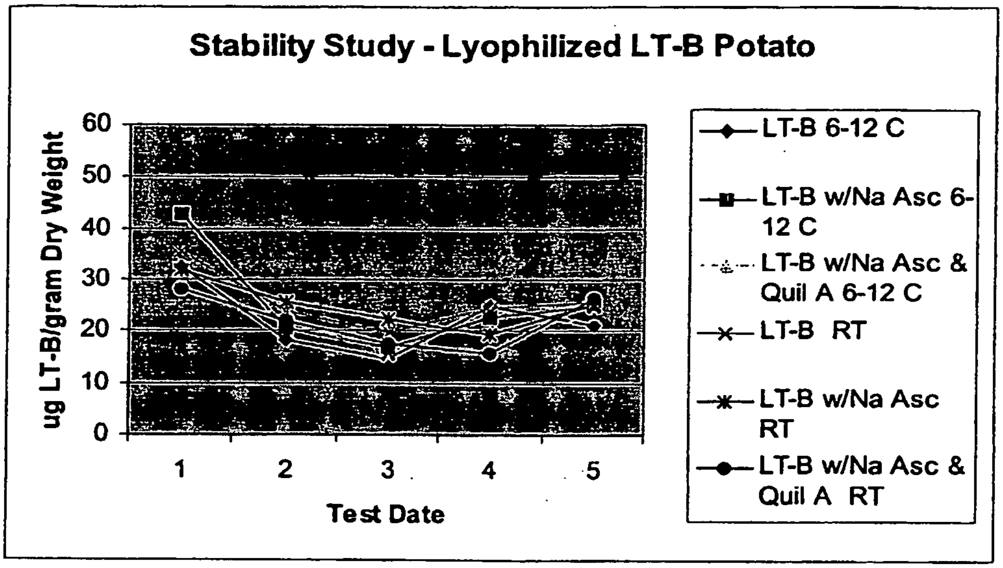

Stability Study - Lyophilized LT-B Potato
ug LT-B/gram Dry Weight
60
50
40
30
20
10
0
1
2
3
4
5
Test Date
LT-B 6-12 C
LT-B w/Na Asc 6-12 C
LT-B w/Na Asc & Quil A 6-12 C
LT-B RT
LT-B w/Na Asc RT
LT-B w/Na Asc & Quil A RT

FIGURE 14b

HA Storage Experiment

Lyoph NT-1 Cells (HAO)

Date	Storage Conditions	ELISA Results
27-Sep-01	(-18°C)	62.35
27-Sep-01	(R.T.)	66.5
25-Oct-01	(-18°C)	88.65
25-Oct-01	(R.T.)	105.33
27-Nov-01	(-18°C)	45.9
27-Nov-01	(R.T.)	60.0
11-Jan-01	(-18°C)	179.3
11-Jan-01	(R.T.)	187.5
8-Mar-02	(-18°C)	* 108.1
8-Mar-02	(R.T.)	* 149.8

*Began using a new lot of Chicken anit-AIV (T/W/68) from SEPRL as the detector antibody in the ELISA

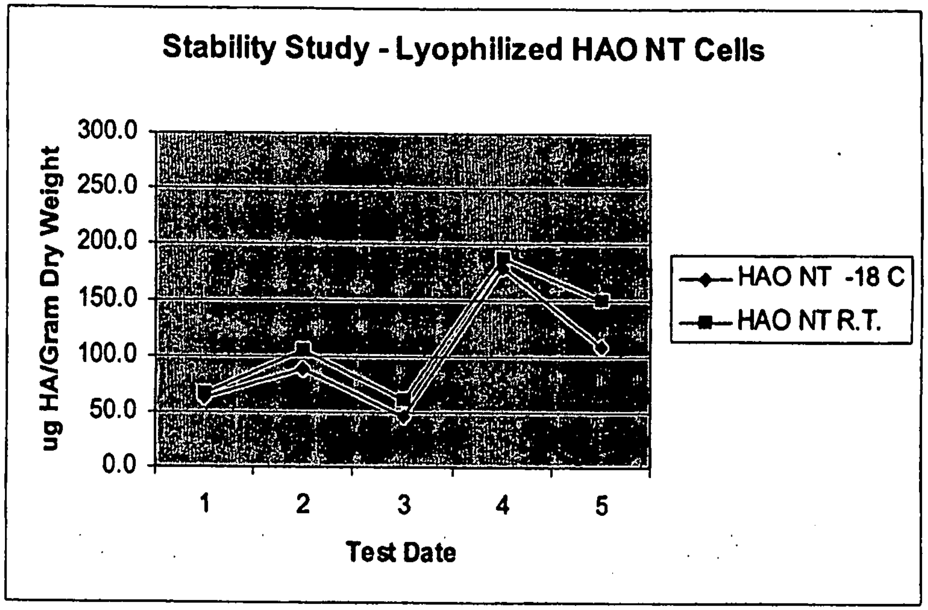


FIGURE 15

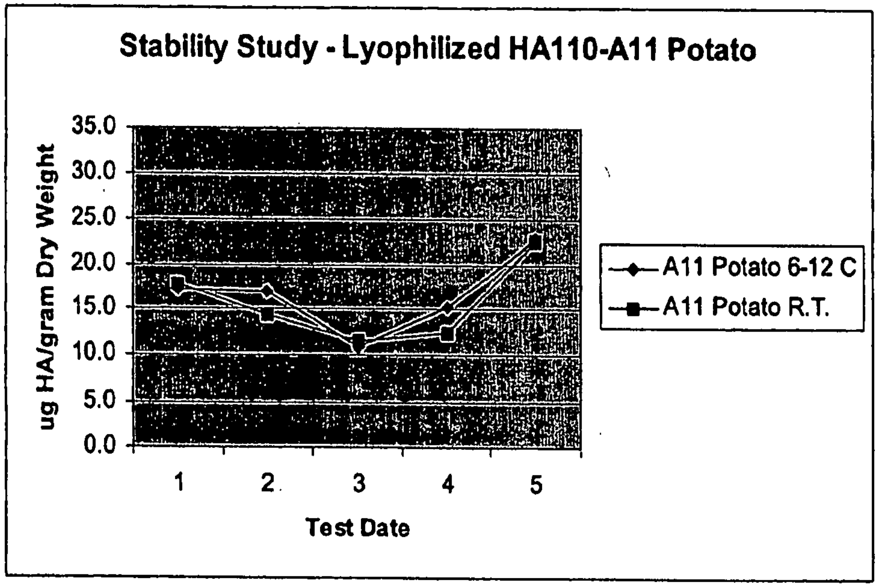
HA Storage Experiment

FIGURE 16

Lyoph HA 110-A11 Potatoes

Date	Storage Conditions	EILISA Results
27-Sep-01	(6-12°C)	17.22
27-Sep-01	(R.T.)	17.74
25-Oct-01	(6-12°C)	16.89
25-Oct-01	(R.T.)	14.41
27-Nov-01	(6-12°C)	11.0
27-Nov-01	(R.T.)	11.5
11-Jan-01	(6-12°C)	15.2
11-Jan-01	(R.T.)	12.4
8-Mar-02	(6-12°C)	* 22.8
8-Mar-02	(R.T.)	* 22.6

*Began using a new lot of Chicken anit-AIV (T/W/68) from SEPRL as the detector antibody in the ELISA



Stability Study

Lyoph NT-1 Cells (SLT102)			
Date	Storage Conditions	LT ELISA Results^A	LT-B-ELISA Results^A
12-Oct-01	(-18°C)	41.4	183.4
12-Oct-01	(R.T.)	41.4	183.4
28-Nov-01	(-18°C)	32	188.8
28-Nov-01	(R.T.)	36.2	201.1
12-Jan-02	(-18°C)	* 18.7	166.1
12-Jan-02	(R.T.)	* 20.6	162.2
13-Feb-02	(-18°C)	11	143.1
13-Feb-02	(R.T.)	12.9	116.3
14-Mar-02	(-18°C)	20.6	188.9
14-Mar-02	(R.T.)	19.9	168.8

A=> Results reported as ug LT or LT-B per gram dry weight

*=> Unknown samples were not parallel to the reference. But Slope ration was close to being acceptable.

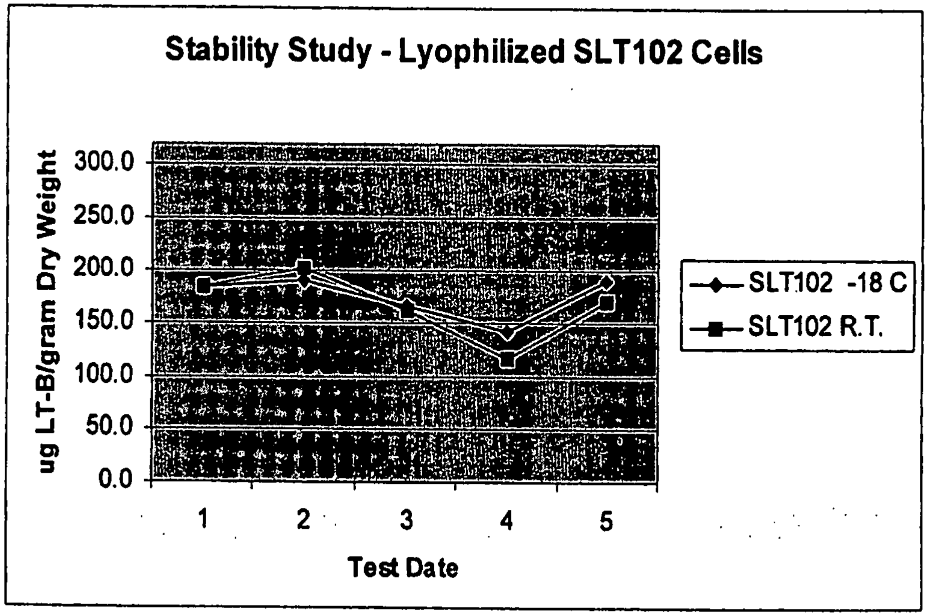


Figure 17